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Deamination of Deoxycytidine Nucleotides by the Obligate Intracytoplasmic Bacterium *Rickettsia prowazekii*

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Thymidylate biosynthesis via the methylation of dUMP is required for DNA replication in *Rickettsia prowazekii*, an obligate intracytoplasmic bacterium. In theory, dUMP synthesis could occur either by the deamination of deoxycytidine nucleotides or by the reduction of uridine nucleotides. Accordingly, the incorporation of both radiolabeled cytidine and uridine into the thymidylate of *R. prowazekii* was examined. After DNA hydrolysis and high-performance liquid chromatography, it was determined that 85% of the rickettsial thymidylate was derived from cytidine and the remaining 15% was derived from uridine. These findings were supported by the identification of a dCTP deaminase activity in extracts of *R. prowazekii*. Extracts of *R. prowazekii* deaminated 1.7 ± 0.3 nmol of dCTP/min/mg of protein (a value calculated to suffice for rickettsial growth), and no measurable activity was observed with dCMP as the substrate.

Members of the genus *Rickettsia* are obligate intracytoplasmic parasitic bacteria. *Rickettsia prowazekii*, the etiological agent of epidemic typhus, is a morphologically typical gram-negative bacterium (1, 2, 8). *R. prowazekii* grows free in the cytoplasm of its eucaryotic host cell unbounded by any internal membranes of host cell origin. This rich external milieu, the eucaryotic cytoplasm, offers many intermediate and end products of metabolism to the bacterium for transport. Carrier-mediated membrane transport systems in *R. prowazekii* have been described for lysine (16), proline (22), UDP-glucose (23), AMP (3), K^+ (21), ATP/ADP (20), and NAD (4). While transport plays a significant role in the acquisition of many rickettsial metabolites, *R. prowazekii* has the ability to synthesize other metabolites. A prime example of this is the synthesis of polyamines from arginine (17).

dUMP can be synthesized from both uridine and cytidine nucleotides. The former route has been previously identified in *R. prowazekii* (6, 18). The latter route for dUMP synthesis, namely deoxycytidine nucleotide deamination catalyzed either by a dCTP deaminase or a dCMP deaminase (9), is the subject of this study. In *Escherichia coli* and *Salmonella typhimurium*, 75% of the cellular dTTP is derived from cytidine and 25% is derived from uracil (12, 13). To date, dCTP deaminases have been found in the gram-negative bacteria *E. coli* (13) and *S. typhimurium* (12), whereas, dCMP deaminases have been found in gram-positive bacteria (7), fungi (14), and protozoan parasites (15).

Incorporation of ribonucleotides into rickettsial thymidylate. The incorporation of ribonucleotides into rickettsial thymidylate was examined by adding both [2-^{14}C]cytidine and [5,6-^{3}H]uridine to the rickettsia-infected host cells. These ribonucleosides are converted in the host cell's cytoplasm to labeled ribonucleotides which are transported by the rickettsiae. The experimental protocol used for these studies has been described previously (18). Briefly, 10^7 Urd$^-$A cells (a uridine-requiring mutant of Chinese hamster ovary cells [CHO-K1]) were infected with rickettsiae, and at 24 h postinfection, 2.5 μCi of [2-^{14}C]cytidine and 5 μCi of [5,6-^{3}H]uridine were added to the medium. At 48 h postinfection, the rickettsiae were isolated from their host cells and the rickettsial nucleic acids were precipitated by placing the samples at −20°C overnight after the addition of 2.5 volumes of ethanol and 1/10 volume of sodium acetate. The rickettsial RNA was hydrolyzed by placing the samples at 50°C for 1 h in 2 N NaOH, and the radioactivity in the hydrolyzed RNA was counted by liquid scintillation spectroscopy after removal of the DNA by precipitation. The rickettsial DNA was hydrolyzed by incubating the samples at 170°C for 30 min after the addition of 88% formic acid, and the labeled thymine was isolated by high-performance liquid chromatography and quantified by liquid scintillation spectroscopy.

Incorporation of both uridine and cytidine into rickettsial thymidylate was demonstrable. *R. prowazekii*-infected Urd$^-$A cells incorporated 1,478 ± 222 dpm of [5,6-^{3}H]uridine/10^7 cells into thymidylate, while mock-infected cells incorporated only 158 ± 13 dpm of [5,6-^{3}H]uridine/10^7 cells into thymidylate (Table 1). Strikingly, *R. prowazekii*-infected Urd$^-$A cells incorporated 16,193 ± 1,106 dpm of [2-^{14}C]cytidine/10^7 cells into thymidylate, while mock-infected Urd$^-$A cells incorporated 525 ± 85 dpm of [2-^{14}C]cytidine into thymidylate.

The relative specific activities of the cytidylates and uridylates were assessed by their incorporation into rickettsial RNA (Table 1). The ratio of radioactive cytidylate to radioactive uridylate in rickettsial RNA was 1.03. If we assume that rickettsiae, like *E. coli* (11), have in their RNA a molar ratio of cytidylate to uridylate of 1.0, then the specific activities of these nucleotides are approximately equal. In contrast, we found in the rickettsial DNA that the ratio of radioactive thymidylate derived from radioactive cytidine to that derived from radioactive uridine was approximately 12. Since half of the label in dUMP is lost in its conversion to dTMP, this ratio suggests that six times more cytidine than uridine is converted to thymidylate. That is, 85% of the rickettsial thymidylate was derived from cytidine and 15% was derived from uridine. These findings are consistent with those published for both *E. coli* and *S. typhimurium* (12, 13).

dCTP deaminase activity in Renografin-purified extracts of *R. prowazekii*. To incorporate cytidine into thymidylate, a

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TABLE 1. Incorporation of cytidine and uridine into RNA and the thymidine of DNA of *R. prowazekii*

R. prowazekii cells	Incorporation into:					
	R. prowazekii RNA			Thymidylate in *R. prowazekii* DNA		
	[^{3}H]uridine	[^{14}C]cytidine	C/U ratio[a]	[^{3}H]uridine	[^{14}C]cytidine	C/U ratio
Infected	187.0 ± 17.2[b]	196.6 ± 10.2		1.5 ± 0.2	16.2 ± 1.1	
Uninfected	4.4 ± 0.5	8.1 ± 0.4		0.2 ± 0.1	0.5 ± 0.1	
Difference[c]	182.6 ± 16.7	188.5 ± 9.8	1.03	1.3 ± 0.2	157.7 ± 1.0	11.87 (5.93)

[a] The ratio of the radioactivity derived from cytidine to that derived from uridine. A corrected ratio, which is required because the tritium in the fifth position of uridine is lost as water in the thymidylate synthase reaction, is shown in parentheses for thymidylate.
[b] Mean kdpm ± standard error for at least two independent experiments.
[c] Value for infected cells minus value for uninfected cells.

dCMP or dCTP deaminase is required. To determine if such activities are measurable in *R. prowazekii*, the methods of Tomita and Takahashi (19) and Beck et al. (5) were used. Briefly, an extract of Renografin-purified *R. prowazekii*, propagated in chick yolk sacs, was prepared as previously described (18). The rickettsial extract (4 mg of protein) was suspended in 1 ml of a reaction mixture containing either 250 μM $MgCl_2$, 1 mM dCTP, and 25 mM Tris-HCl (pH 7.5) or 125 μM $MgCl_2$, 1 mM dCMP, and 25 mM Tris-HCl (pH 7.5). The decrease in A_{280} at 15 and 30 min was measured in 0.1-ml samples after they were mixed with 1 ml of 1 M HCl. The measurement of the disappearance of dCTP or dCMP was based on a difference in the molar extinction coefficients between deoxycytidine and deoxyuridine compounds of 3.41 × 10^3 at pH 7.5.

Measurable dCTP deaminase activity was detectable in extracts of both *R. prowazekii* and the positive control, *E. coli*. No dCMP deaminase activity was demonstrable in either extract. Extracts of *R. prowazekii* deaminated 1.7 ± 0.3 nmol of dCTP/min/mg of protein, and extracts of *E. coli* deaminated 63 ± 19 nmol of dCTP/min/mg of protein. The amount of dCTP deaminase activity measured in broken cell extracts of *R. prowazekii* would be adequate to support an 8- to 10-h doubling time. About 300 pmol of cytidine would have to be deaminated/min/mg of protein to support rickettsial growth if it is assumed that the *R. prowazekii* genome has a size of 1.1 × 10^9 daltons (10) and is 71% A+T and that 85% of the required thymidylate is derived from cytidine via a dCTP deaminase.

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